

The High Resolution X-ray Microscope, XM-1

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Abstract. We give an overview of the activities at the high-resolution x-ray microscope XM-1 at the Advanced Light Source, including both scientific programs and instrumental enhancements. The instrument is being actively used in many fields including biology, environmental and material sciences. A new high efficiency condenser zone plate and precision computer control of the microscope allow users to obtain many hundreds of images in a day. Further developments at XM-1 include a cryogenic sample stage for sample preservation and plans for the implementation of a cryo-tilt stage to capture stereoscopic information.

THE MICROSCOPE

The XM-1 x-ray microscope was built in 1994 by the Center for X-ray Optics (CXRO) at Lawrence Berkeley National Laboratory to provide a high throughput of high spatial resolution transmission images, from a wide variety of thick (< 10 micron) samples (1). Much of the heritage of XM-1 is derived from the microscope pioneered by the University of Göttingen as they share similar optical configurations (2). An overview of XM-1, which is modeled after a conventional bright field microscope is presented in figure 1. Bending magnet radiation from the Advanced Light Source (ALS) provides the illumination source. Radiation from the bending magnet is reflected off of a plane mirror at glancing incidence to filter out higher photon energies. The effective microscope operating range in photon energy is 250 - 900 eV.

The condenser zone plate (CZP), which collects the radiation from the ALS, is used to illuminate the sample. A new CZP constructed using electron beam lithography

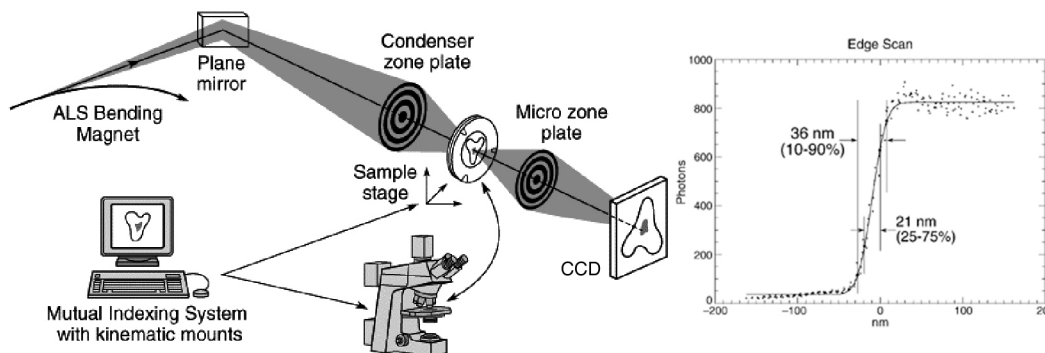


FIGURE 1. a) Optical Layout of the Xm-1 X-ray Microscope b) knife edge resolution test results.

was installed in October, 1998 (3). The new CZP has a diameter of 9 mm, 41,000 zones, an outer zone width of 55 nm, and much-improved efficiency. The combination of the CZP and a pinhole produces a linear monochromator of moderate spectral resolution by exploiting the known chromatic aberrations of zone plates. Sample illumination wavelength is selected by varying the distance between the CZP and pinhole. Following the pinhole, the beam is brought through a thin window to atmospheric pressure and to the sample holder.

Samples with a thickness of less than 10 microns can be imaged dry, hydrated, or cryogenically fixed. The sample can be mounted between nominal 1000Å thick silicon nitride windows, or on standard TEM grids. The sample holder has a three-point kinematic mount designed to fit both the XM-1 and a custom Zeiss Axioplan visible light microscope (VLM). Sample positions and focus are pre-selected with the VLM, which is mutually indexed with the sample stage of XM-1. X-Y position accuracy is typically 2 microns over a 3mm field with focal accuracy better than 1 micron.

This system is especially useful in reducing total exposure times, as the researcher is able to pre-select individual sample elements with the visible light microscope, followed by automated x-ray imaging with accurate position and focus. Moreover, the researcher is able to view many samples in one session, thus providing a high throughput with hundreds to even thousands of images obtainable per day.

The radiation passing through the sample is collected by the micro zone plate (MZIP) and imaged at high magnification onto a 1024 x 1024 pixel x-ray CCD camera. Images are typically magnified 2400x. The pixel size projected back to the sample is typically 10 nm. There is an option to combine several pixels into one by binning the CCD camera, thus reducing the radiation dose and time exposure, for example a factor of 4 with 2 x 2 binning. Exposure times for images with low photon noise are typically on the order of 1 second. Images and microscope parameters are recorded digitally and are available to the user within seconds of exposure.

To first order, the spatial resolution of the microscope is determined by the outer zone width of the MZIP (although other factors enter). The present MZIP has an outer zone width of 35 nm with 318 zones and a diameter of 45 microns (3). In knife-edge measurements, the 10% to 90% intensity range, which approximates Rayleigh resolution, yields a result of 36 nm (Figure 1). Results are expected to improve in the coming months. A new 25 nm outer zone width MZIP is being installed as this article goes to press.

Though the CZP illuminates a 10-micron field of view, it is possible to build images of a larger area using a montage assembly. This automated process builds a larger image based on several sub-field images. Typical images range in dimensions up to 100 x 100 microns. Using cross correlation techniques the smaller images are placed at the proper positions creating a nearly seamless montage.

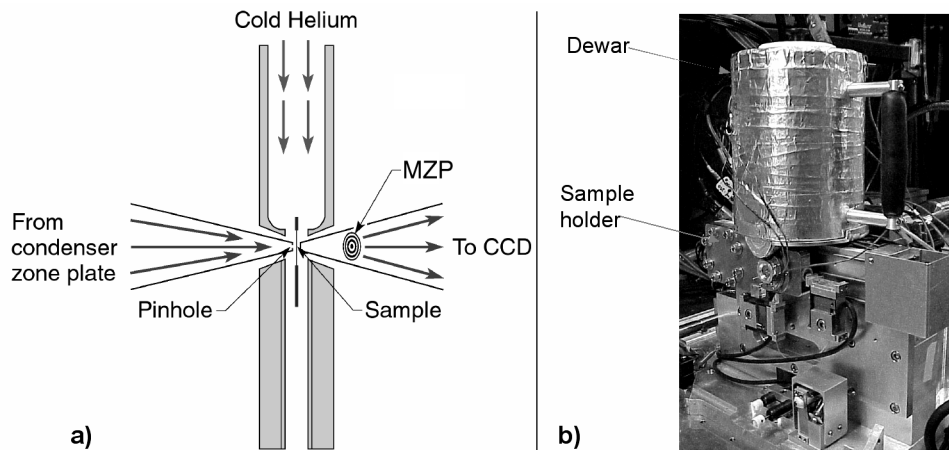


FIGURE 2. Cryogenic fixation system (a) schematic (b) sample stage.

Cryogenic Sample Stage

In order to preserve the structural integrity of biological samples, a cryogenic sample holder has been built. The radiation dose from each exposure at XM-1 is roughly 10^7 Gray which, depending on the sample, can cause changes in the morphology of wet samples after one or two images. In order to keep the samples intact for many images, and also to prevent the formation of ice crystals, the sample is quickly frozen at a rate of about 3000 K/s to a temperature of -130°C where it is maintained. The freezing of the sample is accomplished by blowing cold helium gas across the sample (figure 2). The helium is cooled as it passes through a dewar filled with liquid nitrogen, and then is directed across the sample window to freeze the sample. Once the sample is frozen, it is able to withstand many exposures. In one experiment a cryogenically fixed 3T3 fibroblast cell was imaged 40 times in the same location (figure 3). At the resolution of this microscope there was no apparent change in the nuclear membrane due to the multiple exposures.

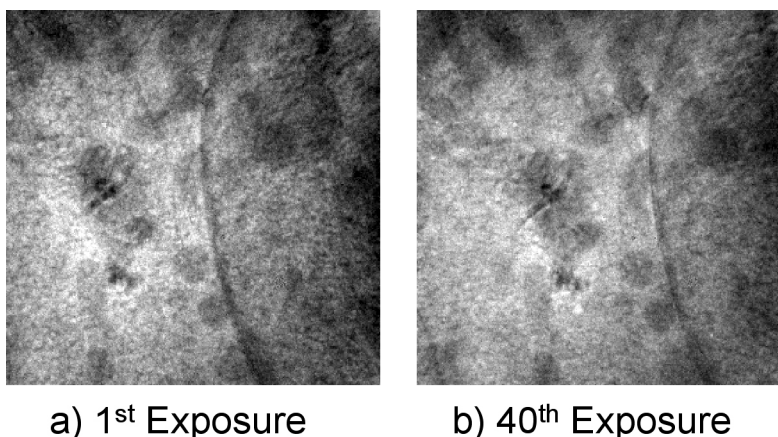


FIGURE 3. Cryogenically Fixed 3T3 Cells after a) 1st and b) 40th Exposures. The thin vertical line is the nuclear membrane which shows no observable modification due to the radiation dose. (with C. Larabell, D. Yager, unpublished)

Spectromicroscopy

As mentioned previously, the sample illumination photon energy is selectable by varying the distance between the condenser zone plate and pinhole. With recent additions to the microscope control software it is now relatively easy to obtain a series of full field sample images, with each image at a different energy. A series of images taken at 100 different photon energies each with a spatial resolution of about 36 nm takes about 20 minutes. In order to measure the spectral resolution of XM-1, the Calibration and Standards Beamline (6.3.2) at the ALS (4) which has a known spectral resolution of $\lambda/\Delta\lambda > 4000$ was used to precisely determine the spectral dependence of absorption of a thin sample of CaF_2 at the Calcium L edge (~ 350 eV). The same measurement of the sample was also performed with XM-1. The convolution of the high precision 6.3.2 data with spectral bandwidth of XM-1 yields the measured spectra from XM-1. The result of this calculation yielded a spectral resolution of 0.5 eV FWHM indicating a spectral resolving power of $(E/\Delta E)$ approximately 700 at 350 eV. Such a resolution is sufficient to distinguish different elemental species. In some cases it is sufficient to facilitate chemical state identification. It has been shown in a test sample to show that it is possible to distinguish between the different oxidation states of chromium (5).

SCIENTIFIC HIGHLIGHTS

In recent years many exciting scientific studies have been conducted making use of XM-1. In the biological arena, high-resolution protein localization has been demonstrated (6). Gold-labeled antibodies were used to selectively identify proteins and oligonucleotides within a cell. The gold particles were silver-enhanced to form aggregates approximately 50 nm in diameter. These particles were resolved with the XM-1 and a mapping of the location of the target proteins and oligonucleotides were obtained. Some of the proteins and oligonucleotides labeled and imaged are microtubulin, nuclear pore complex, and actin mRNA. An example of the images is shown in figure 4, where tubulin protein labeled within a 3T3 epithelial cell is clearly seen. More recently, cryo-fixed images of a 3T3 cell were obtained as seen in figure 5 (6). In the montage assembly, the nuclear membrane, nucleoli, organelles, and granules are all clearly visible.

In addition, many material and environmental science studies were conducted using XM-1. The microscope has been used to study Alkali-silica reactions in concrete samples used in various water storage dams (7). These reactions can cause swelling and cracking of the dam structure. Reaction gel morphology was studied extensively with XM-1 (figure 6a) The microscope has also been used to observe the distribution of Mn in micronodules produced by biomineralization (8). Making use of the image contrast above and below the Mn L_3 edge provided the mechanism to produce a mapping of the Mn distribution. Moreover the high spatial resolution of the XM-1 was able to reveal a network of very fine needle like shaped structures.

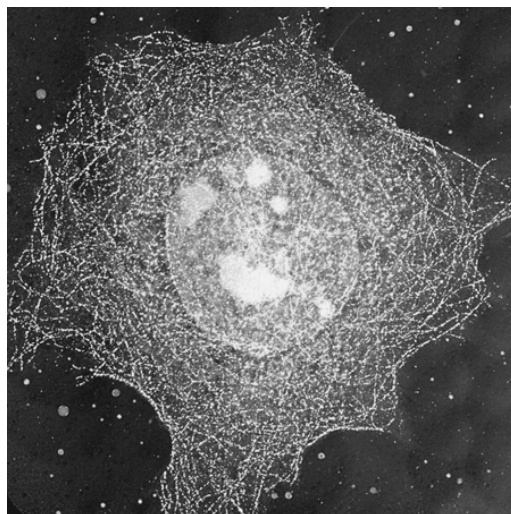


FIGURE 4. Tubulin Network in Epithelial Cell (C. Larabell, S. Lelievre, D. Hamamoto, M. Bissell, A. Nair and W. Meyer-Ilse, submitted for publication)

The microscope was also used to study macromolecular structures of humic substances in aqueous solutions, soils, and in sediments (figure 6b) (9). These studies have shown that the macromolecular structures vary as a function of both solution chemical composition and mineral chemistry. This information is useful in accurately predicting the organo-mineral interactions, C-cycle, and contaminant transport in soils and aquatic systems. The above is just a sampling of studies using XM-1 during the past year. Additional studies involved nanocrystals, chromium-reducing bacteria, and magnetic materials.

Conclusion

A user-friendly, full field, x-ray transmission imaging microscope has been constructed and is in use for a wide variety of scientific studies at a spatial resolution of 36 nm. Users are able to obtain a set of images of their specimens, which can be dry, hydrated, or cryo-fixed. Plans are underway to improve the spatial resolution of the microscope and to develop its spectromicroscopic capabilities.

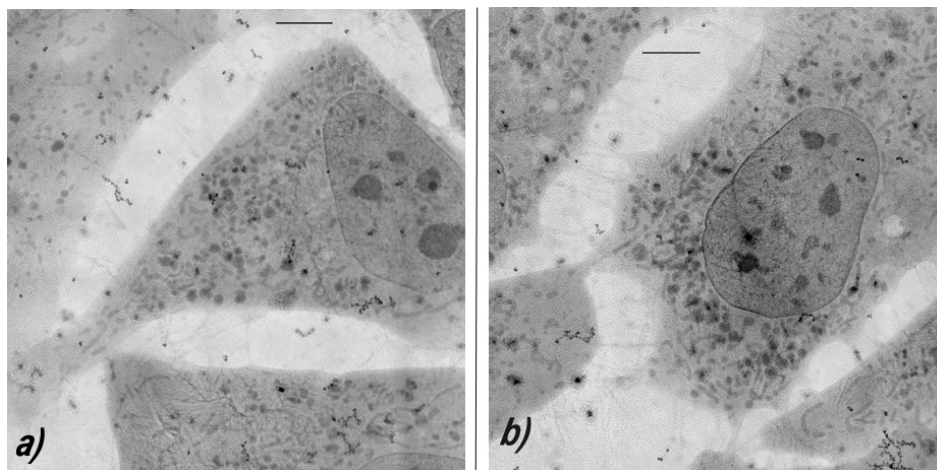


FIGURE 5. Cryogenically Fixed 3T3 Cells (with C. Larabell, D. Yager, T. Shin, these proceedings)

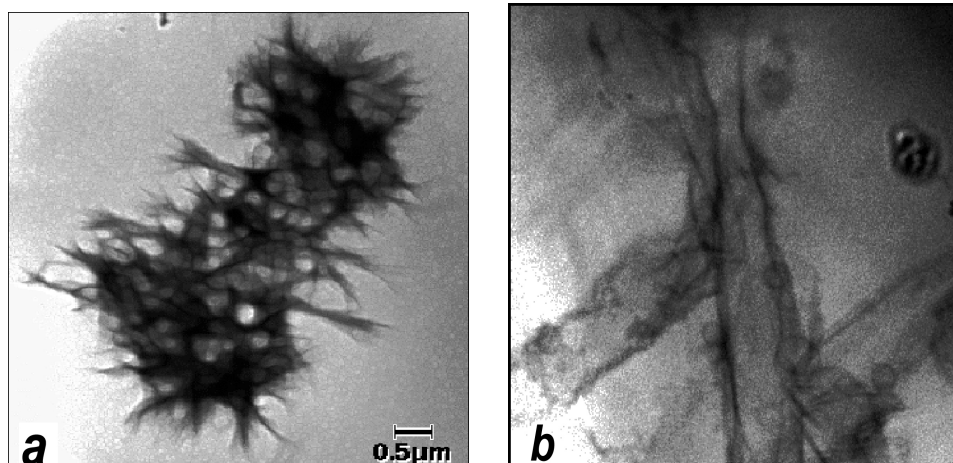


FIGURE 6. a) Alkali-silica reactions (K. Kurtis et al., ref 7) b) Humic substances in aqueous solutions (S. Myneni, et al, ref 9)

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